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Active streaming in actomyosin solutions

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SUMMARY

Solutions of actomyosin, extracted from plasmodia of *Physarum polycephalum* or from rabbit's striated muscle, were introduced into microcapillaries in the presence of ATP. Vigorous and sometimes oscillatory streaming was observed which resembled in many respects cytoplasmatic streaming.

It is now well established that the mechanical force generated in a stimulated striated muscle originates from the interaction between actin-containing and myosin-containing filaments involving the splitting of ATP. This force is capable of moving the two sets of filaments past each other so that the effect observed externally is the shortening (or "contraction") of the muscle. The presence of actin and myosin has been demonstrated in smooth muscles as well as in many different non-muscular cells. It is now generally assumed that ATP splitting by an actomyosin system is the energy source which creates the driving force for cytoplasmatic streaming. This assumption originates from the belief that there exists a basic mechanism common to all cellular movements and from the analogy between the response of cytoplasmatic streaming on the one hand and of the actomyosin system *in vitro* on the other hand to the action of various factors such as the addition of ATP, the presence of Ca^{2+}, changes in pH, the application of various other reagents such as sulphydryl reagents, changes in temperature and hydrostatic pressure *etc.* (*cf.* Kamiya[1]). In addition, one may account for the rate of cytoplasmatic streaming and the magnitude of the motive forces involved in the cellular movements on the basis of the assumption that actin and myosin filaments, similar to those of striated muscle, are responsible for the streaming[2,3].

A wide variety of theories has been put forward for the explanation of cytoplasmatic streaming. Since more is known about muscular contraction than any other motile system, it so happened that concepts of muscular contraction have been

automatically applied in the study of other cell movements. The very application of the term "contraction" to non-muscular cells implies that streaming is caused by the "contraction" or "shrinkage" of a structured system. Some theories assume that by the contraction of a gel phase an hydrostatic pressure difference is created which drives the cytoplasm; other theories suggest that forces are generated on cell membranes to which "contractile" filaments are anchored (similarly to the z-bands in a striated muscle). While the primary force-generating mechanochemical event involving the splitting of an ATP molecule is probably common to the various actomyosin systems, the assembly of many actin and myosin active sites to form "super structures" differs from one motile organelle to another according to function and stage of development. The formation of intricate insoluble structures does not seem to be essential for the very generation of force but for its amplification and directivity. The "extrapolation" from the highly developed striated muscles to primitive systems such as the amoeba runs contrary to the arrow of time and is therefore quite dangerous.

One may wonder whether the interaction between a free single (*i.e.* unbound) actin filament in solution with free myosin (either in the form of filaments or as single molecules) cannot create mechanical impulses[4] which might cause the movement of the protein species relative to each other and to the surrounding fluid. This "streaming" may cause gel→sol transformations in vicinal regions. "Contraction" will thus be an outcome of the active streaming rather than its cause.

We were tempted to examine the possibility that interaction between actin, myosin and ATP in solution is indeed capable of inducing streaming, *i.e.* just to prepare solutions containing action, myosin and ATP and look for conditions under which streaming might be exhibited.

In order to achieve massive streaming which could be visualized say under the microscope it is obvious that the region observed should contain many actin filaments, most of which are oriented in a certain direction. It has been observed[5] that at sufficiently high concentrations F-actin filaments form an anisotropic phase by parallel orientation. The orientation in a bulk phase differs from one point to another. It makes sense to believe that the orientation of the filaments will be the same throughout a capillary if its width is not too large in comparison to the length of the filaments. The orientation might also depend on the protein concentration and on environmental factors. In the following we shall describe experiments in which actomyosin solutions containing ATP were introduced (by suction) into microcapillaries and checked for streaming.

Glass capillaries of 20–100 μm internal diameter and 0.5–3.0 cm long were utilized. At both ends, the capillaries were wider (about 1 mm internal diameter). Liquid occupied 0.5–2.0 cm at each of the wide parts.

We used myosin B which was isolated from microplasmodia of the myxomycete *Physarum polycephalum*. The reasons for using this actomyosin were that cytoplasmatic streaming in *Physarum* is very intensive and has been thoroughly investigated[1] and that the actin and myosin of this system are well characterized[6,7]. The microplasmodia were cultured at 23 °C in shaken flasks containing semi-defined medium[8], kindly supplied by

Dr I. Het. The plasmodia from 250 ml of the culture solution (30 h after inoculation) were allowed to sediment in a 250-ml measuring tube in the cold (all subsequent operations were carried out in ice). After pouring the supernatant the tubes were filled with a cold 60 mM KCl solution and the sediment (25 ml) re-suspended. The washings were repeated three times. Mucous material was removed by treatment with 100 ml of a 60 mM KCl, 20 mM EDTA solution (pH adjusted to 7.0) for 30 min with gentle stirring. This treatment caused a loss of coloring material into the supernatant and fading out of the cells but the form of the cells and the cytoplasmic streaming were not affected. To the final sediment (30 ml) 10 ml of the following solution were added in order to extract the myosin: 2 M KCl, 0.08 M EDTA, 5 mM ATP, pH 8.2, to give a final concentration of 0.5 M KCl, 20 mM EDTA and 1.25 mM ATP. From this point the procedure of Hatano and Ohnuma[6] was followed except that precipitation by dilution was carried out only once and this was followed by extraction with a smaller volume (1 ml) of a lower ionic strength (0.35 M KCl) solution. These changes were introduced in order to minimize protein loss and to get a final solution (to be used for the streaming experiments) with a relatively high protein concentration and an ionic strength which is close to the physiological[2]. 0.1 ml of a solution containing 5 mg/ml myosin B in 0.35 M KCl was added to an Mg-ATP solution to a given final solution containing 0.4 mg/ml of myosin B, 30 mM KCl, 4 mM Mg-ATP, 16 mM phosphate or imidazole buffer, pH 7.0. Immediately after mixing the myosin B with the ATP solution a sample was drawn into a capillary. Observation under the microscope (magnification 150–250) revealed a very intensive movement of suspended particles in the narrow middle part at a rate of about 1 mm/s which is comparable to the rate *in vivo*. Movement continued in one direction for about 1 min then stopped abruptly for about 10 s during which time the particles appeared to be moving very slowly in the opposite direction. Then, all of a sudden, the particles began moving vigorously in the original direction. The meniscus of the liquid in the wider ends of the capillary moved much more slowly in the same direction as the particles in the narrow part. This seems to indicate that the particles are passively dragged by the streaming fluid. The above "cycle" repeated itself 4–5 times and afterwards movement proceeded continuously at a lower rate in the same direction for at least half an hour. The magnitude of the maximal velocity observed and the periodic nature of the streaming at the beginning cannot be ascribed to viscoelastic streaming due to the suction of the solution into the capillary and are reminiscent of streaming in micro-capillary strands of intact *Physarum*. Streaming was found to continue in the same direction even against an opposing hydrostatic pressure difference of up to 4 mm water created by tilting the capillary.

During the later stages one could detect an increase in the number of granular particles. It seems that at least part of the particles observed at the beginning of the experiment are composed of insoluble myosin B. Samples taken from the same myosin B–ATP solution (kept in the cold) half an hour after the mixing still exhibited movement but this was much less intensive. At the same time the number of particles was larger. The stock solution appeared to have undergone superprecipitation. It is interesting to notice that at the later stages of the observation, when the number of particles (presumably

originating from superprecipitation) was much larger than during the first half hour, the velocity of streaming became much slower. This, and the fact that movement always took place horizontally, seem to indicate that streaming does not originate from convection currents induced by the sedimentation of these particles.

Myosin B which was kept in the cold for 5 days in 0.35 M KCl preserved its capability to induce movement immediately after adding ATP. The above experiments were repeated with several preparations of myosin B from *Physarum*, giving similar results.

The next step was obviously to repeat the same experiments with myosin B from muscle. Myosin B was extracted from the back muscle of a rabbit[9]. The solution tested contained 1 mg/ml myosin B, 0.75 M KCl, 20 mM Tris–maleate buffer, pH 7.6, 8 mM $MgCl_2$, 0.1 mM $CaCl_2$, 3 mM ATP. Immediately after mixing the myosin B (in 0.5 M KCl) with the other components we could again observe intensive movement of particles (about 1 mm/s) which was maintained continuously in one direction for 20 min. Application of an opposing hydrostatic pressure could not stop or change the direction of the movement unless it was greater than about 3 mm water. After 20 min, movement stopped abruptly and this was immediately followed by bulk superprecipitation. No movement could be detected during superprecipitation or afterwards.

In order to prolong the "clearing phase" which precedes superprecipitation so that movement could be maintained for longer periods and without interference caused by superprecipitation we added a regenerative system which kept a constant level of ATP for a relatively long time. To the above described system containing myosin B from muscle we added 5 mM phosphoenolpyruvate and 50 μg/ml pyruvate kinase. The ATP concentration was 0.5 mM. Movement now continued for much longer periods and superprecipitation appeared much later (after about 1 h). The particles now moved forwards and backwards in an oscillatory manner resembling in this sense cytoplasmatic streaming in intact *Physarum* and in a sap obtained from *Physarum* by homogenization and centrifugation (Tirosh, R., Oplatka, A. and Het, I., submitted to *J. Cell Biol.*). The period of these oscillations was not regular and the movement was not as vigorous as in the absence of the regenerative system. We then noticed that the oscillation became more regular if the internal diameter of the thin part of the capillary was smaller than 50 μm, its length around 2 cm, while the volume of solution occupying the wider ends was relatively small. Thus, when a solution containing 1 mg/ml myosin B, 40 mM KCl, 0.1 M Tris–HCl buffer, pH 7.3, 8 mM $MgCl_2$, 0.1 mM $CaCl_2$, 5 mM ATP, 3 mM phosphoenolpyruvate, 50 μg/ml pyruvate kinase was introduced into a capillary 3 cm long with an internal diameter of 30 μm, we observed 5 subsequent cycles of 4 min each, followed by streaming in one direction until it stopped.

It is interesting to note that the simple system of actomyosin in solution exhibits oscillatory streaming which is one of the most intriguing characteristics of cytoplasmatic streaming.

Since the mechanochemical force in striated muscle is believed to be generated as the result of the interaction of the ATP-carrying heavy meromyosin subfragment-1 (S-1) subunits of the myosin molecules we were naturally tempted to examine streaming

in a capillary containing actin, ATP and either heavy meromyosin or S-1 subunits. The conditions were similar to those utilized in the case of myosin B. The heavy meromyosin and the actin concentrations were varied between 0.5–5.0 mg/ml and 0.2–1.0 mg/ml, respectively. Since the ATPase activity of heavy meromyosin–actin decreases with increasing ionic strength[10] we worked in the range of 10–50 mM KCl. The results until now have been ambiguous and further experiments are being carried out.

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